ELECTROPHORETIC GEL FORMING SYSTEM

BACKGROUND OF THE INVENTION

This invention relates to methods and apparatus for producing electrophoretic gels which require polymerization of material during their formation.

The analysis of complex biochemical systems is dependent on technology which allows individual molecules to be separated. Standard techniques exist which allow protein, DNA, and RNA molecules to be separated and/or purified on the basis of their size, electrical charge, physical structure or a combination thereof. Most of these techniques employ at least one type of gel electrophoresis. In gel electrophoresis, the molecules to be separated are subjected to an electric field which causes them to enter a gel of a known pore size, charge and pH. Depending on the conditions, different molecules will be either retarded or accelerated as they pass through the gel, and hence separated.

One of the most common materials used for electrophoresis is polyacrylamide. A polyacrylamide gel is formed by the chemical cross-linking of a monomer, acrylamide, with a co-monomer, such as N,N'-methylenebisacrylamide (BIS), N,N'-bisacrylylcystamine (BAC), N,N'-(1,2-dihydroxyethylene) bisacrylamide (DHEBA), or N,N'-diallyltartardiamide (DATD). The ratio, as well as the concentration of these monomers determines the final pore size, and hence sieving properties of the gel. Chemical cross-linking, or polymerization, of the gel is effected by the formation of free radicals by an initiator, such as ammonium persulfate (APS) or riboflavin. Polymerization is made even more efficient by the addition of an accelerator such as N,N,N',N'-tetramethylethylenediamine (TEMED) or 3-dimethylaminoproprionitrile (DMAPN).

Non-acrylamide chemically cross-linked matrices have recently come to market. See e.g., AT Biochem, Inc. catalog. These are prepared in a manner similar to polyacrylamide gels.

Generally, an electrophoretic gel is prepared in the following manner. While wearing gloves, and typically in a fume hood, the user prepares the monomer and co-monomer as concentrated aqueous stock solutions which are stored at 4° C. until use. An initiator such as APS is prepared on the day of use as an aqueous solution and stored at 4° C. Accelerators such as TEMED are provided as liquids. Other components of the gel depend on the type of molecules to be separated. For example, for one-dimensional separation of proteins, water, Tris(hydroxymethyl)methylamine (TRIS) and sodium dodecyl sulfate (SDS) are usually prepared as aqueous stock solutions. These four stock solutions are then mixed to prepare a final liquid which is then poured into a suitable apparatus and allowed to polymerize to form a polyacrylamide gel.

SUMMARY OF THE INVENTION

The present invention concerns an apparatus and method for overcoming a long standing problem in the gel forming field. Acrylamide is a potent neurotoxin, and chronic exposure to this chemical in either solid or liquid form results in a well recognized clinical condition characterized by a large fiber neuropathy and sensory ataxia. Other chemicals used to form polyacrylamide gels, such as BIS, APS, BAC, DMAPN and TEMED, are either known toxins or have undetermined safety risks. The present invention allows a gel to be formed without need for pipetting or handling of toxic solutions, while maintaining flexibility in the structure of the final polymerized gel. Moreover, the apparatus so described has a long potential shelf-life, and can be adapted for use in separation of proteins, DNA, and RNA.

The distinct advantages of this system over conventional methods are as follows: the apparatus and method of its use minimizes contact with acrylamide, or its equivalent, and other known toxins; allows gel preparation to be accomplished by a complete novice in very little time; minimizes time required for gel preparation by eliminating the need to prepare at least four different stock solutions; potentially increases reproducibility of gel preparation; has a long potential shelf life; and is good for the environment since extensive disposable plasticware and washable glassware are no longer required to prepare the resulting polyacrylamide gel.

Thus, in a first aspect, the invention features an apparatus for forming an electrophoretic gel which includes a polymer, e.g., polyacrylamide. The apparatus has at least two sealed compartments with inner volumes separated from each other by at least one burstable seal. One inner volume contains a monomer which can be polymerized to form the polymer, the other inner volume includes a catalyst, such as an accelerator or initiator. Bursting of the burstable seal allows the monomer and catalyst to contact each other and, in conjunction with any other necessary components held within the apparatus, to form a liquid medium in the apparatus suitable for forming the desired gel.

The burstable seal is chosen from one of many well known in the art such that it is broken, and the relevant components mixed, only when suitable external pressure is applied. The whole apparatus, of course, includes an outer layer of non-burstable material which encloses all the necessary chemical components. By monomer is meant to include chemicals such as acrylamide which copolymerize with other monomers, e.g., BIS, to form the final polymeric gel. By catalyst is meant to include accelerators and initiators alone and in combination.

In a related aspect, the invention features an apparatus with at least two sealed compartments, each having separate inner volumes. The apparatus includes all of the chemical components necessary to form the electrophoretic gel. One or more of those components is provided in one inner volume and one or more of the components is provided in the other inner volume. The inner volumes are separated from each other by at least one burstable seal. Bursting of the burstable seal allows contact of the two inner volumes and thereby contact of the components held within those inner volumes. When all the components are contacted within the apparatus a liquid medium for forming the electrophoretic gel is provided.

In yet another related aspect, the invention features apparatus with four compartments separated by burstable seals. In each compartment is a monomer, initiator, accelerator or liquid buffer. The seals can be broken to allow mixing of the contents of each compartment, and thereby production of the desired liquid medium to form a polymeric gel.

In preferred embodiments, the apparatus includes a co-monomer which co-polymerizes with the monomer to form the polymer, e.g., the co-monomer is provided in the same inner volume which holds the monomer; the